Supercritical fluid extraction and fractionation applicated to borage seeds

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ABSTRACT

A study of the fractionation of the borage oil obtained with supercritical fluid extraction is presented. The values of the acidity index, antioxidant capacity and composition in fatty acid are measured. The results obtained indicate than a fractionation of the oil allow the decrease the value of the acidity index obtaining good values of the antioxidant capacity in comparison with other oils. Nevertheless, the fractionation does not achieve an increase in the antioxidant capacity and a separation of the fatty acids. The conditions selected to realized the fractionation was 90 bar and 40ºC in the first separator

INTRODUCTION

Borage (*Borago officinalis*) is a plant native from Asia; nowadays, the main producers are the United Kingdom, the Netherlands, New Zealand and Canada. Borage seed contains 30% of oil and this oil presents one of the highest amounts of γ-linolenic (n-6) and other n-6 essential fatty acids. The γ-linolenic is the first intermediate formed during the conversion of linolenic acid to prostaglandins, very important to be present in many reactions in the human body. This omega-6 acid has anti-inflammatory, antithrombotic and anti-carcinogenic properties. Also, it has been used for treating arthritis, certain skin problems (i.e., atopic eczema), reproductive disorders, including breast pain and premenstrual syndrome, cardiovascular diseases and neurological problems related to diabetes [1, 2].

Vegetable oil from seeds is traditionally produced by hexane extraction from ground seeds. The process is very efficient, but its major problem is represented by hexane elimination after extraction. The possible thermal degradation of the oil and the incomplete hexane elimination (from 500 to 1000 ppm residue) are the drawbacks of this process. Therefore, several authors have proposed the substitution of the traditional process by SC-CO_2 extraction of oil from seeds [3]. Indeed, triglycerides forming seed oils are readily soluble in SC-CO_2 at 40 °C and at pressures larger than about 280 bar.

Extraction of compounds from natural sources is the most widely studied application of supercritical fluids (SCFs) with several hundreds of published scientific papers. Indeed, supercritical fluids extraction (SFE) has immediate advantages over traditional extraction techniques: it is a flexible process due to the possibility of continuous modulation of the

solvent power/selectivity of the SCF, allows the elimination of polluting organic solvents and of the expensive post-processing of the extracts for solvent elimination.
Several compounds have been examined as SFE solvents. However, carbon dioxide (CO_2) is the most popular SFE solvent because it is safe, readily available and has a low cost. It allows supercritical operations at relatively low pressures and at near-room temperatures. The only serious drawback of SFE is the higher investment costs if compared to traditional atmospheric pressure extraction techniques. However, the base process scheme (extraction plus separation) is relatively cheap and very simple to be scaled up to industrial scale.
More sophisticated extraction schemes contain two or more separators. In this case, it is possible to fractionate the extract in two or more fractions of different composition by setting opportune temperatures and pressures in the separators.
The objective of the present work was to apply supercritical technology using carbon dioxide as the solvent in the production of oil from borage seeds. A method for the fractionation of the extract using precipitation in different cyclone separators was subsequently analyzed.

MATERIALS AND METHODS

Samples and chemicals

Borage seeds were supplied by Institute of Sustainable Agriculture, CSIC, Cordoba, Spain. The carbon dioxide (99.995%) used was provided by Air Liquids (Barcelona, Spain). Standard of methyl ester and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was provided by Sigma–Aldrich and the other reagents (ethanol, toluene, potassium hydroxide 0.1 mol/l (0.1N) ethanolic SV, phenolphthalein solution, ethyl acetate, methanol) by Panreac.

Extraction and fractionation at high pressure

The extraction and fractionations were carried out in equipment supplied by Thar Technology (Pittsburgh, PA, USA, model SF2000) provided with an extraction vessel (capacity of 2 l) and a pump with a maximum flow rate of 150 g/min of carbon dioxide. Two cyclonic separators allowed periodic discharge of the extracted material during the SFE process. A pressure-regulating valve that controlled the pressure allowed different conditions to be applied to the separators. The equipment was also fitted with a solvent recycling system. The extraction conditions in the extractor vessel were 55 ºC and 400 bar. These conditions were optimized in a previous work [4]. The extract obtained in the extraction process, was separated in two separators connected in series. Four sets of fractionation conditions were investigated:

- *Extraction 1*: separator 1 (S1) at 200 bar-45 ºC and separator 2 (S2) at atmospheric conditions.
- *Extraction 2*: separator 1 (S1) at 100 bar-40 ºC and separator 2 (S2) at atmospheric conditions.
- *Extraction 3*: separator 1 (S1) at 90 bar-40 ºC and separator 2 (S2) at atmospheric conditions.
- *Extraction 4*: separator 1 (S1) at 70 bar-40 ºC and separator 2 (S2) at atmospheric conditions.

The fractions were collected in the two cyclonic separators and stored at 4 ºC with the exclusion of light.
The experiments on each sample were carried out in duplicate in order to evaluate the variability of the measurements.

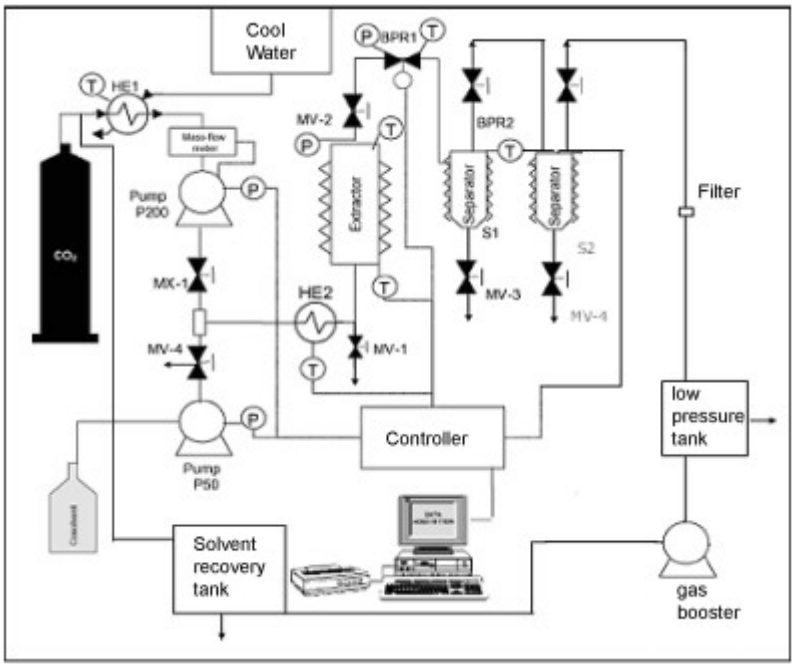

Figure. 1 Schematic diagram of the equipment.

Composition of fatty acids

Compositions of fatty acids of two separator has been carried out in a gas chromatograph from Agilent Technologies model 6890N with a capillary column model TR-CN100 (60 m of longitude x 0.25 mm of internal diameter x 0.20 μm of thickness) and detector of flame ionization. Injector and detector temperature were 280 ºC and 260 ºC respectivily. The oven temperature was 185 ºC at the rate of 38.02 cm/s. The carrier gas was hydrogen and air and hydrogen as auxiliary gases. The extracts obtained were treated to be transformed in fatty acid methyl ester (FAME) [5]. A typical chromatogram of the FAME is presented in figure 2.

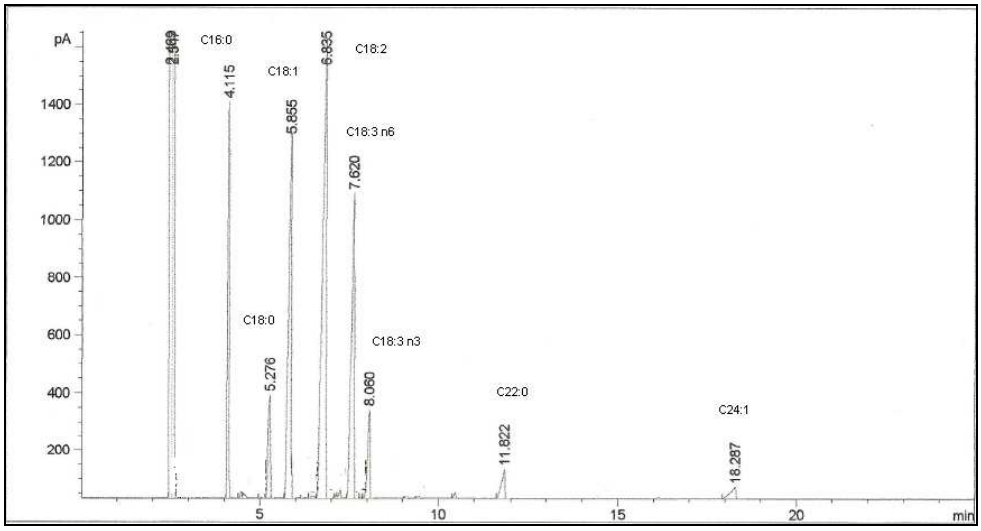

Figure 2. Typical chromatogram of FAME obtained from borage oil.

Acidity index

Acidity index of fractions was analyzed, according to the Official European Community method [6]. Acidity index is the mass, in mg, of potassium hydroxide that is necessary to neutralize free fatty acids (FFA) present in 1 g of sample. It is usual to represent such as percentage of oleic acid, that it is the most abundant FFA.

Antiradical activities of potent antioxidants by DPPH

The antioxidant activities were determined using DPPH as a free radical. For each fractionation conditions, different concentrations were tested (expressed as the mg of oil/ mg DPPH*). Oil solution in ethyl acetate (0.1mL) was added to 3.9mL of a 6×10^{-5}mol/L ethyl acetate DPPH* solution. The decrease in absorbance was determined at 515 nm at different times until the reaction "reached a plateau". The exact initial DPPH* concentration (CDPPH*) in the reaction medium was calculated by linear regression from a calibration curve with the equation,

$$Abs515nm = -0.0065 \times (CDPPH^*)\ 29.3112 \qquad (1)$$

For each extraction conditions the percentage of DPPH* remaining at a time of four hours was determinate. From these information, the percentage of DPPH* remaining at the steady state was determined and the values transferred onto another graph showing the percentage of residual DPPH* at the steady state as a function of the weigh ratio of antioxidant to DPPH*. Antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH* concentration by 50% (Efficient Concentration = EC_{50} (mg oil/ mg DPPH*) [7].

RESULTS

Fractionation of the extracts

The process of fractionation of the extracts involved the stepwise precipitation of the extracts obtained in the extraction process. This was achieved by modification of the density of CO_2 through changes in the pressure and temperature in the separator. In this way the compounds that were least soluble in the supercritical solvent precipitated in the first separator while the more soluble materials remained in the second separator.

Figure 3 shows the results of extraction yields for the extracts obtained at 200, 100, and 90 bar. In this case of the fractionation realized at 70 bar and 40ºC one only extracts has been obtained. In this case do not fractionation in two separators. For 200 bar/ 45 ºC the percentage of extract obtained in separator 1 was low. In extraction 2 and 3, the yield from separator 1 increased to 55, 25 and 81, 87 % respectively.

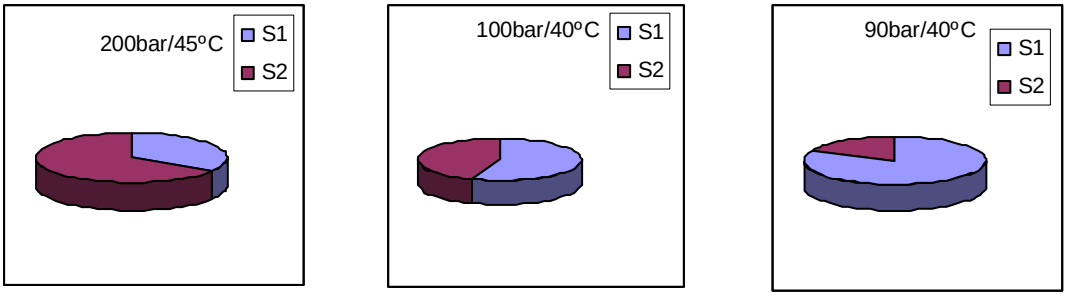

Figure. 3. Percentage separation of the extract in the two separators.

Figure 4 shows the fatty acids compositions of the extracts obtained in both separators and the composition of the extract obtained in a soxhlet extraction using hexane as solvent. There are no important differences in the composition obtained in the different extracts. These means that the fraction of the supercritical process do not extract selectively different fatty acids and practically, all of this compounds has the same solubility in the supercritical fluid.

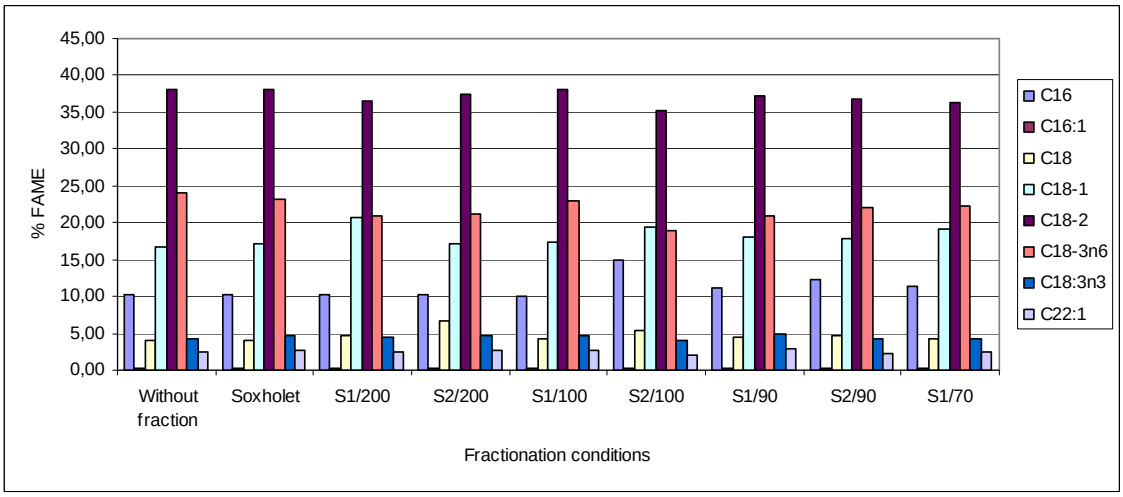

Figure. 4. Composition of fatty acids of the different fractions obtained.

In figure 5, we represented the acidity index of the different fractions obtained in in two separators and the acidity obtained in the soxhlet extraction. We can see that the highest variations of acidity index in the extract are produced in separator 2 at 90 bar and 40 ºC. The variations obtained in the acidity index of the fraction separated in the separator 1 are very low. Nevertheless, the value obtained is lower than the value of acidity index obtained in the oil extracted without fractionation (5, 37 %). These results indicate than the free fatty acids have a greater solubility in the solvents and go on solubilized in carbon dioxide until the separator 2.

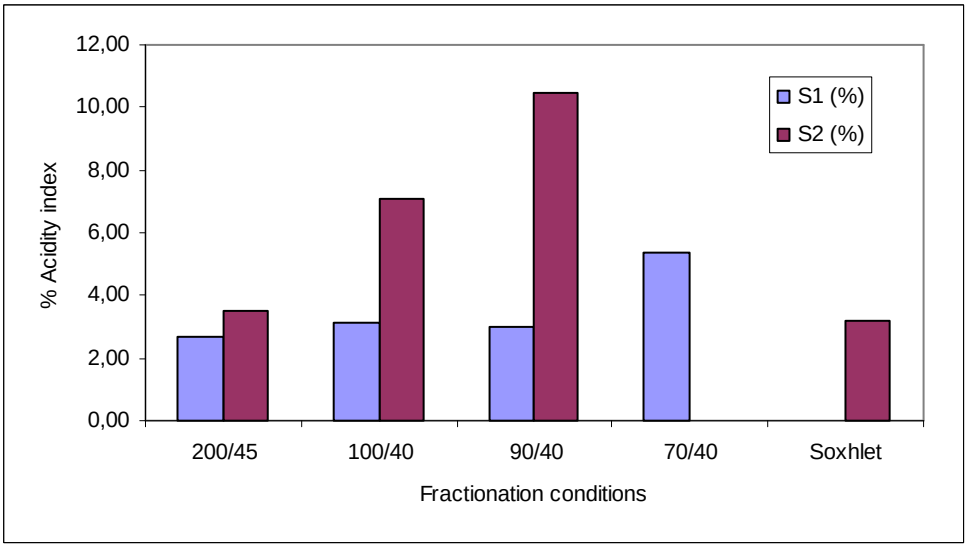

Figure.5. Acidity index of extracts obtained in two separators at different fractionations conditions.

Finally, we have determinate the antioxidant activities using DPPH methods as a free radical. The results obtained are presented in Table 1 join to data of the antioxidant capacity of other oils obtained from the bibliography [8], and other compounds with important values of this parameter.

Table1. Antioxidant capacity of fractions oil measured by DPPH methods (EC_{50} values, mg/mg DPPH)

	Separator 1	Separator 2
200bar/45ºC	150	178
100bar/40ºC	132	182
90bar/40ºC	138	162
without fractionation	118	
Soxhlet	228	
Walnut oil	1514,3	
Almond oil	712,2	
Hazelnut oil	478,5	
Peanut oil	1395,9	
Pistachio oil	377,9	

A lower value of the EC_{50} means that the sample has higher value of the antioxidant capacity. The data obtained shown small differences in both separators. The highest values of the antioxidant capacity are found always in the first separator vs the second separator. Nevertheless, the lowest value of the EC_{50} was found when the oil obtained is not fractionated. From these results, a fractionation of the oil is not necessary if the objective of the process is to obtain oil with a high antioxidant capacity.
The EC_{50} value of soxhlet extraction is higher that supercritical extraction. Therefore, the highest values (lower value of the EC_{50}) of the antioxidant capacity are found always in extraction with supercritical fluid.
Comparing the data obtained with those obtained from the literature, we can conclude that the borage oil present a higher value of the antioxidant capacity than other oils.

CONCLUSION

The supercritical fractionation of borage oil allows obtaining oil with low values of acidity index and acceptable values of antioxidant capacity. The high amount of γ-linolenic acid and the values of this antioxidant capacity make this oil a good choice for human consumption. The best results were obtained when we fractionate the oil in the first separator at 90 bar and 40ºC. Nevertheless, this fractionation decreases mildly the value of the antioxidant capacity in comparison of the supercritical extraction of the oil without fractionation.

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